

Cytochrome *o* (*cyoABCDE*) and *d* (*cydAB*) Oxidase Gene Expression in *Escherichia coli* Is Regulated by Oxygen, pH, and the *fnr* Gene Product

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The aerobic respiratory chain of *Escherichia coli* contains two terminal oxidases that catalyze the oxidation of ubiquinol-8 and the reduction of oxygen to water. They are the cytochrome *o* oxidase complex encoded by *cyoABCDE* and the cytochrome *d* oxidase complex encoded by *cydAB*. To determine how these genes are regulated in response to a variety of environmental stimuli, including oxygen, we examined their expression by using *lacZ* protein fusions in wild-type and *fnr* mutant strains of *E. coli*. Anaerobic growth resulted in a 140-fold repression of *cyoA*'-'*lacZ* expression relative to aerobic growth and a 3-fold increase in *cydA*'-'*lacZ* expression. Anaerobic repression of both fusions was mediated in part by the *fnr* gene product, as evidenced by a 30-fold derepression of *cyoA*'-'*lacZ* expression and a 4-fold derepression of *cydA*'-'*lacZ* expression in an *fnr* deletion strain. Supplying wild-type *fnr* in *trans* restored wild-type repression for both fusions. *Fnr* thus functions as an anaerobic repressor of both *cyoABCDE* and *cydAB* expression. Reduced-minus-oxidized difference spectrum analyses of cell membranes confirmed the effect of the *fnr* gene product on the production of cytochrome *d* oxidase in the cell. Based on the pattern of anaerobic *cydAB* expression observed, we propose the existence of a second, as yet unidentified, regulatory element that must function either to activate *cydAB* expression as oxygen becomes limiting or to repress *cydAB* expression aerobically. Whereas cytochrome *o* oxidase encoded by *cyoABCDE* appears to be produced only under oxygen-rich growth conditions, in keeping with its biochemical properties, cytochrome *d* oxidase is expressed moderately aerobically and is elevated yet further when oxygen becomes limiting so that the organism can cope better under oxygen starvation conditions. We also examined *cyoABCDE* and *cydAB* expression in response to growth on alternative carbon compounds and to changes in the culture medium pH and osmolarity.

Escherichia coli contains two distinct cytochrome oxidases that catalyze the oxidation of ubiquinol-8 to allow cellular respiration with oxygen as the terminal electron acceptor (1). These membrane-bound enzymes are the cytochrome *o* oxidase complex, encoded by *cyoABCDE*, and the cytochrome *d* oxidase complex, encoded by *cydAB* (2, 10). The cytochrome *o* oxidase complex contains two protoheme IX groups (designated cytochrome *b*₅₅₅ and *b*₅₆₂) plus two copper atoms per complex (15, 19). The cytochrome *d* oxidase contains three heme prosthetic groups in three spectrally distinct cytochrome components. These include two protoheme IX moieties (designated cytochrome *b*₅₅₈ and *b*₅₉₅) and one chlorin of the cytochrome *d* type. There are no copper or nonheme iron centers present in this enzyme (16, 20). The two enzymes differ in their affinity for oxygen and their sensitivity to respiratory inhibitors. Previous studies indicate that the cytochrome *o* oxidase complex is the predominant enzyme under oxygen-rich growth conditions, whereas the cytochrome *d* oxidase complex, which has higher affinity for oxygen, accumulates as oxygen becomes limiting (17, 22).

The *cyoABCDE* and *cydAB* genes map to 10.2 and 16.7 min, respectively, on the *E. coli* map (2, 10). The genes for each have been cloned, and the *cydAB* genes have been sequenced (9). Georgiou et al. have shown that *cydAB* expression increases as cultures enter the late-logarithmic or

stationary phase (6). They suggested this was due to a decrease in oxygen tension in the culture medium as a result of increased cell density, although regulation of *cydAB* expression in response to carbon substrate, pH, or cell growth rate could not be distinguished from the response to oxygen. They demonstrated that the *fnr* gene product, a transcriptional activator of many anaerobically induced respiratory genes, was not required for transcriptional activation of *cydAB* expression (6). To date, little is known about expression of the *cyoABCDE* genes.

To rigorously examine the regulation of the cytochrome oxidase genes in response to oxygen availability and other environmental stimuli, we analyzed the expression of *cyoA*'-'*lacZ* and *cydA*'-'*lacZ* protein fusions that were contained in a single copy on the *E. coli* chromosome. We found both operons to be highly regulated in response to oxygen, and we established the role of the *fnr* gene product in this process. The effect of anaerobiosis and *Fnr* on synthesis of the cytochrome *d* oxidase complex was confirmed by reduced-minus-oxidized difference spectrum analysis. We also examined the effects of carbon source, pH, and medium osmolarity on *cyoABCDE* and *cydAB* expression.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids. The genotypes of *E. coli* K-12 strains MC4100 (23) and SM1 (S. B. Melville and R. P. Gunsalus, J. Biol. Chem., in press), plasmids pfnr2 and pfnr3 (14), and bacteriophage λGC101 (6) have been described previously.

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Construction of *cyoA'*-*lacZ* and *cydA'*-*lacZ* fusion strains. A specialized lambda phage, λ VLH114, containing an in-frame gene fusion between the 24th codon of *cyoA* and the 9th codon of *lacZ* was constructed as follows. Plasmid pRG110, which contains *cyoABCDE*, was digested with *Hind*III and then with *Bal*31 exonuclease. The mixture was made blunt ended with Klenow fragment and ligated with *Bam*HI linkers by using T4 DNA ligase. The ligation mixture was then digested with *Bam*HI and *Pvu*II and ligated into pMLB1034 (23) that had been cut with *Bam*HI and *Sma*I. pVLH114 was isolated by its ability to confer a Lac⁺ phenotype, and the fusion junction between *cyoA* and *lacZ* was determined by sequence analysis. The fusion was then transferred to λ RZ5 by standard techniques (23). The *cyd'*-*lacZ* protein fusion phage λ GC101 has been described (6). High-titer lysates containing λ VLH114 or λ GC101 were used to lysogenize MC4100 (23); single lysogens were isolated and designated MC4100(λ VLH114) and MC4100(λ GC101). By similar methods, λ VLH114 and λ GC101 were introduced in a single copy into the chromosome of the *fnr* deletion strain SM1. In all strains the wild-type *cyoABCDE* and *cydAB* loci were preserved intact, since the *cyoA'*-*lacZ* and *cydA'*-*lacZ* fusion phages integrate at the lambda attachment site on the chromosome.

Cell growth. For strain manipulations and maintenance, cells were grown in Luria broth or on solid medium. When required, ampicillin, chloramphenicol, and 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside were added to the medium at concentrations of 80, 30, and 40 mg/liter, respectively. For the β -galactosidase assay, cells were grown in glucose (40 mM) minimal medium (pH 7.0) (13) unless otherwise indicated. For assay of cells grown on other carbon sources, 40 mM glycerol, sorbitol, xylose, gluconic acid, succinate, or lactate was substituted for the glucose. Aerobic and anaerobic growth conditions were as previously described (3). High aeration of cultures during aerobic growth was accomplished by shaking 15-ml culture volumes in 150-ml flasks. The use of larger flasks to increase aeration had no additional effect on *cyoA'*-*lacZ* or *cydA'*-*lacZ* expression. To determine the effect of pH on *cyoA'*-*lacZ* and *cydA'*-*lacZ* expression, phosphate-buffered medium was adjusted to pH 6.5, 7.0, or 7.5 at 37°C. For the lower pH range, 2-(*N*-morpholine)ethanesulfonic acid-buffered medium was used (27). The medium pH was adjusted to 5.3, 5.8, or 6.35 at 37°C before sterilization. The pH of the culture medium was measured before and after cell growth; it dropped by no more than 0.2 pH unit during the experiment. The pH values reported in Fig. 2 were for the media before cell inoculation. To determine whether *cyoA'*-*lacZ* and *cydA'*-*lacZ* were regulated by changes in medium osmolarity, the salt concentration in the medium was adjusted to 0, 0.15 or 0.3 M NaCl as indicated below.

β -Galactosidase assay. β -Galactosidase assays were performed as previously described (3). β -Galactosidase values represent the averages of at least four experiments with variations of no more than 10% from the mean.

Difference spectra. Reduced-minus-oxidized difference spectra were recorded on an Aminco DW-2a dual-beam spectrophotometer with sodium dithionite and potassium ferricyanide as the reductant and the oxidant, respectively. Cells were grown in minimal medium (pH 7.0) or L broth, to an optical density at 600 nm of 1.2, harvested by centrifugation, suspended in sodium phosphate buffer (100 mM, pH 7.0), and then broken by passage through a French pressure cell at 6,000 lb/in². Extracts were diluted such that each

TABLE 1. Effect of oxygen on *cyoA'*-*lacZ* and *cydA'*-*lacZ* expression in wild-type and *fnr* strains

| Strain ^a | β -Galactosidase activity ^b | | Regulation (fold) ^c |
|--|--|-----------------|--------------------------------|
| | +O ₂ | −O ₂ | |
| <i>cyoA'</i> - <i>'lacZ</i> | | | |
| MC4100(λ VLH114) (wild type) | 156 | 1.1 | 142 |
| SM1(λ LH114) (<i>fnr</i>) | 148 | 35 | 4 |
| SM1(λ VLH114)(<i>pfnr</i> 3) (<i>fnr</i> ⁺) | 140 | 1.4 | 100 |
| <i>cydA'</i> - <i>'lacZ</i> | | | |
| MC4100(λ GC101) (wild type) | 104 | 311 | 3 |
| SM1(λ GC101) (<i>fnr</i>) | 107 | 1,254 | 12 |
| SM1(λ GC101)(<i>pfnr</i> 3) (<i>fnr</i> ⁺) | 119 | 141 | 1 |

^a Cells were grown on glucose minimal medium aerobically or anaerobically as described in the text.

^b Units are given as nanomoles of *ortho*-nitrophenyl- β -D-galactopyranoside hydrolyzed per minute per milligram of protein.

^c Obtained by dividing the aerobic value by the anaerobic value for the *cyoA'*-*lacZ* strains and by dividing the anaerobic value by the aerobic value for the *cydA'*-*lacZ* strains.

cuvette contained 5 mg of total protein per ml. Protein assays were performed by the method of Peterson (21).

Materials. *ortho*-Nitrophenyl- β -D-galactopyranoside, 2-(*N*-morpholine)ethanesulfonic acid, and ampicillin were purchased from Sigma Chemical Co., St. Louis, Mo. 5-Bromo-4-chloro-3-indolyl- β -D-galactopyranoside was obtained from International Biotechnologies, Inc., New Haven, Conn. All other chemicals used were of reagent grade.

RESULTS

Effect of oxygen on cytochrome oxidase gene expression. The effect of oxygen on *cyoA'*-*lacZ* and *cydA'*-*lacZ* expression was determined by measuring β -galactosidase levels in MC4100(λ VLH114) and MC4100(λ GC101) grown in the absence or presence of saturating oxygen (Table 1). *cyoA'*-*lacZ* expression, which was maximal in aerobically grown cells, was reduced over 140-fold in anaerobically grown cells. In contrast, *cydA'*-*lacZ* was expressed at a moderate basal level in aerobically grown cells and expression was increased threefold in response to anaerobiosis.

Effect of *fnr* on *cyoA'*-*lacZ* and *cydA'*-*lacZ* expression. To examine the involvement of the *fnr* gene product in regulation of *cyoABCDE* and *cydAB* gene expression, λ VLH114 and λ GC101 were introduced into the chromosome of the *fnr* deletion strain SM1. *Fnr* has been shown to function as a transcriptional activator of several respiration-associated operons, including those encoding nitrate reductase (26), trimethylamine *N*-oxide:dimethyl sulfide reductase (3), and fumarate reductase (14). Anaerobically, *cyoA'*-*lacZ* expression was 30-fold greater in the *fnr* deletion strain than in the wild-type strain (Table 1). Aerobically, *cyoA'*-*lacZ* expression remained unchanged. Supplying wild-type *fnr* to the *fnr* deletion strain on a multicopy plasmid restored wild-type repression during anaerobic growth (Table 1). Thus, *Fnr* acts to repress *cyoABCDE* expression in response to anaerobiosis.

Interestingly, *fnr* also appears to act as a repressor of *cydAB* expression in response to anaerobiosis. *cydA'*-*lacZ* expression increased 12-fold during anaerobic versus aerobic growth in the *fnr* deletion strain, compared with the 3-fold increase seen in the wild-type strain (Table 1). The increased anaerobic expression of *cydA'*-*lacZ* was eliminated when wild-type *fnr* was supplied to SM1 on a multi-

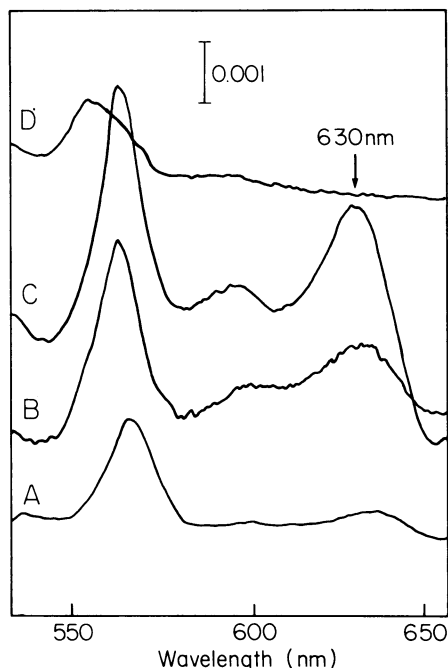


FIG. 1. Reduced-minus-oxidized difference spectra of cell extracts prepared from wild-type and *fnr* mutant strains grown in glucose minimal medium under the following conditions: (A) MC4100 grown aerobically, (B) MC4100 grown anaerobically, (C) SM1 grown anaerobically, (D) SM1(pfnr2) grown anaerobically. The vertical scale is in absorbance units.

copy plasmid. β -Galactosidase activity in the aerobically grown cultures was the same in the Δfnr and wild-type strains.

Reduced-minus-oxidized difference spectra of cell extracts. To determine whether the level of cytochrome *d* in the cell membrane reflects the pattern of *cydA'-lacZ* expression described above, we analyzed the reduced-minus-oxidized difference spectra of wild-type and *fnr* strains grown aerobically or anaerobically. The room-temperature difference spectra of dithionite-reduced minus ferricyanide-oxidized cell extract in the 560- to 650-nm region revealed the presence of the cytochrome *d* oxidase complex due to the distinct chlorinlike heme that absorbs in the region of 630 nm (20). In agreement with the gene fusion data, wild-type cells grown aerobically exhibited a small peak at 630 nm due to absorption of this heme (Fig. 1). This peak was increased in anaerobically grown wild-type cells, was increased further in an anaerobically grown *fnr* deletion mutant, and was nearly absent in the *fnr* deletion strain that contained a multicopy plasmid, pfnr2, with the wild-type *fnr* gene (Fig. 1). When the cytochrome *d* content was estimated by measuring the area of the 630-nm peak, a fourfold higher amount was seen in the MC4100 strain grown anaerobically versus the amount in MC4100 grown aerobically. The Δfnr strain grown anaerobically contained a 14-fold higher level compared with that of the aerobic wild-type strain. Thus, the presence of the cytochrome *d* oxidase complex in the cytoplasmic membrane parallels the pattern of *cydAB* expression as determined by measuring β -galactosidase expression from the *cydA'-lacZ* fusion and supports the proposed role of Fnr as an anaerobic repressor of *cydAB* expression.

Effects of carbon source, pH, and osmotic strength on *cyoA'-lacZ* and *cydA'-lacZ* expression. To determine

TABLE 2. Effect of carbon compounds used for cell growth on *cydA'-lacZ* and *cyoA'-lacZ* expression

| Carbon source ^a | β -Galactosidase activity ^b of: | |
|----------------------------|--|---|
| | MC4100(VLH114) (<i>cyoA'-lacZ</i>) | MC4100(λ GC101) (<i>cydA'-lacZ</i>) |
| Glucose | 144 | 104 |
| Sorbitol | 202 | 129 |
| Xylose | 173 | 108 |
| Gluconic acid | 245 | 229 |
| Glycerol | 231 | 118 |
| Succinate | 307 | 196 |
| Lactate | 412 | 239 |

^a Cells were grown in minimal medium aerobically with the indicated carbon compound added at a final concentration of 40 mM.

^b Units are given as nanomoles of *ortho*-nitrophenyl- β -D-galactopyranoside hydrolyzed per minute per milligram of protein.

whether either *cyoABCDE* or *cydAB* expression varied significantly in response to the carbon source, MC4100 (λ VLH114) and MC4100(λ GC101) were cultured aerobically in minimal medium with glucose, glycerol, sorbitol, xylose, gluconic acid, succinate, or lactate. Expression of *cyoA'-lacZ* and *cydA'-lacZ* was lowest in glucose-grown cells and was not increased in general by more than twofold in cells grown on the alternate carbon compounds (Table 2). The exception was a threefold difference in *cyoA'-lacZ* expression in cells grown on lactate.

To determine the effect of pH on *cyoA'-lacZ* and *cydA'-lacZ* expression, wild-type and *fnr* deletion strains were cultured in media adjusted to various pH values ranging from 5.3 to 7.5 (Fig. 2). In the wild-type strain, *cyoA'-lacZ* expression increased about fourfold during aerobic growth as the pH of the medium increased from 5.5 to 7.5. Little to no change was seen in *cydA'-lacZ* expression under these conditions. Neither *cyoABCDE* nor *cydAB* gene expression varied significantly in the wild-type strain during anaerobic growth. However, in the *fnr* deletion strains grown anaerobically, expression varied greatly (Fig. 2). *cyoA'-lacZ* expression increased over 40-fold in the SM1 Δfnr strain as the pH increased from 5.5 to 7.5. Likewise, *cydA'-lacZ* expression decreased about sixfold as the pH increased from 5.5 to 7.5 in the *fnr* deletion strain.

We also tested the effect of varying the osmolarity of the culture medium on *cyoA'-lacZ* and *cydA'-lacZ* expression. When the medium osmolarity was varied from 50 to 300 mM NaCl, we found no dramatic changes in the expression of either fusion, whether the cells were grown aerobically or anaerobically, although a reproducible 1.5- to 2-fold increase was seen (Table 3).

DISCUSSION

Cytochrome *o* oxidase (*cyoA'-lacZ*) gene expression was maximal in aerobically grown cells and repressed over 140-fold by anaerobiosis to levels that were barely detectable. We have shown that the *fnr* gene product, which is known to function as a transcriptional activator of a number of genes encoding anaerobic respiratory enzymes, functions as a repressor of *cyoA'-lacZ* expression during anaerobic growth (i.e., 30-fold derepression in an *fnr* deletion strain). However, Fnr does not account for all of the observed repression of *cyoABCDE* expression, since *cyoA'-lacZ* expression was still repressed approximately fourfold by anaerobiosis in the Δfnr strain. It was recently proposed that the level of ubiquinol oxidase in the cell is primarily con-

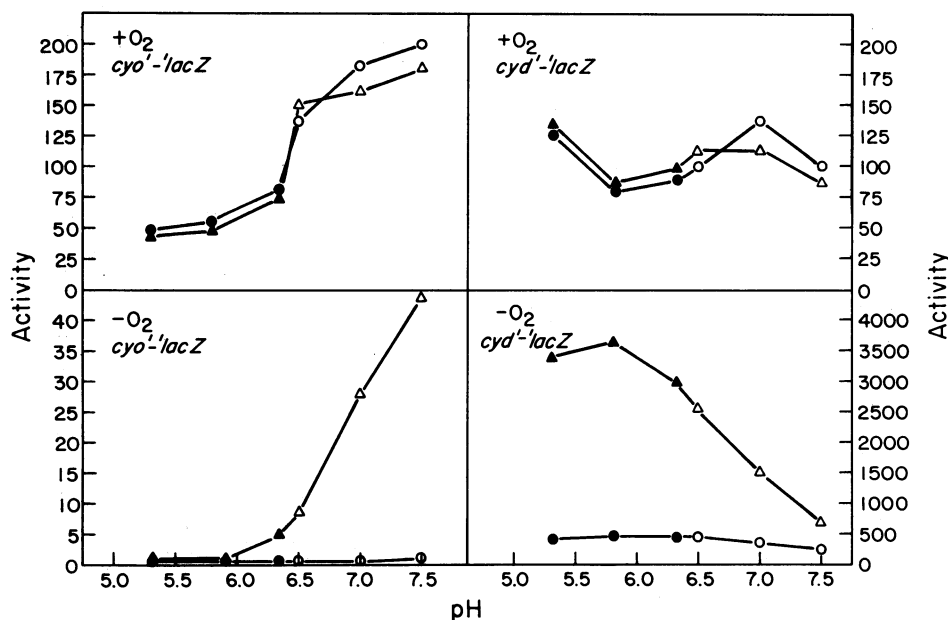


FIG. 2. Effect of pH on *cyoA'*-*lacZ* and *cydA'*-*lacZ* expression. Cells were grown either aerobically or anaerobically in glucose minimal medium adjusted to the indicated pH as described in Materials and Methods. Symbols: Δ and \blacktriangle , Δfnr strains; \circ and \bullet , wild-type strains; \bullet and \blacktriangle , lower-pH-range buffer [2-(*N*-morpholine)ethanesulfonic acid]; \circ and Δ , upper-pH-range phosphate buffer.

trolled by the *arcA* gene product under anaerobic conditions (12). These results are in apparent disagreement with the findings of our study. However, it is likely that the remaining regulation of *cyoABCDE* is due to this gene product, and this possibility is being addressed by others. The greater than 140-fold repression of *cyoABCDE* expression in response to anaerobiosis could, therefore, be fully accounted for by the combined regulation via the *fnr* and *arcA* gene products.

The cytochrome *d* oxidase (*cydA'*-*lacZ*) gene fusion was expressed at a moderate basal level in aerobically grown cells and was elevated about threefold during anaerobic growth. In an *fnr* deletion strain, *cydA'*-*lacZ* was expressed at the same basal level when cells were grown aerobically, but a 12-fold increase was seen in response to anaerobiosis (Table 1). Thus, *fnr* also functions as a repressor of *cydAB* expression under anaerobic growth conditions. That *cydAB*

gene expression in the Δfnr strain is about four times higher than in the wild-type strain indicates that another regulatory gene must also be involved in controlling *cydAB* gene expression. We propose the existence of a second regulatory gene whose product must function either to activate *cydAB* expression as oxygen becomes limiting or, alternatively, to repress *cydAB* gene expression aerobically. It should be noted that this regulator does not appear to function in a manner previously demonstrated for *arcA* (e.g., anaerobic repressor [12]). The existence of this proposed second regulator of *cydAB* expression is currently the focus of our studies.

As determined by reduced-minus-oxidized difference spectra, we have shown that the presence of the cytochrome *d* oxidase complex in the cytoplasmic membrane reflects the pattern of *cydAB* gene expression in response to anaerobiosis and the *fnr* gene product. Although the regulation of the catalytic activity of the cytochrome *d* subunit can be accounted for by transcriptional regulation of the structural genes without invoking posttranslational control. Thus, as for the anaerobic respiratory enzymes, it appears that the regulation of synthesis of the aerobic respiratory enzymes, at least cytochrome *d* oxidase, is primarily at the level of transcription of the structural genes. Our results are in direct contrast with those recently reported by Frey et al., who proposed that *fnr* is required for activation of *cydAB* expression based on the presence and absence of the cytochrome *d* absorption peak at 630 nm in reduced-minus-oxidized difference spectra (5). Since Frey et al. grew the cells in unbuffered L broth before spectral analysis, we also examined cells grown in this way to determine whether the different culture conditions might account for the different results, but we found no difference between the absorption spectra and that shown in Fig. 1 (data not shown). We conclude from both genetic and biochemical data that *Fnr* is not required

TABLE 3. Effect of medium osmolarity on *cyoA'*-*lacZ* and *cydA'*-*lacZ* expression

| Strain ^a | O ₂ | β -Galactosidase activity ^b at an NaCl concn of: | | |
|---|----------------|--|--------|-------|
| | | 0 M | 0.15 M | 0.3 M |
| <i>cyoA'</i> - <i>lacZ</i> | | | | |
| MC4100(λ VLH114) (wild type) | + | 144 | 200 | 188 |
| MC4100(λ VLH114) (wild type) | — | 2 | 5 | 7 |
| SM1(λ VLH114) (Δfnr) | + | 172 | 204 | 224 |
| SM1(λ VLH114) (Δfnr) | — | 66 | 52 | 53 |
| <i>cydA'</i> - <i>lacZ</i> | | | | |
| MC4100(λ GC101) (wild type) | + | 117 | 157 | 176 |
| MC4100(λ GC101) (wild type) | — | 298 | 339 | 396 |
| SM1(λ GC101) (Δfnr) | + | 101 | 151 | 189 |
| SM1(λ GC101) (Δfnr) | — | 803 | 1,276 | 1,454 |

^a Cells were grown on minimal glucose medium with the NaCl concentration adjusted as indicated.

^b Units are given as nanomoles of *ortho*-nitrophenyl- β -D-galactopyranoside hydrolyzed per minute per milligram of protein.

for activation of *cydAB* expression, but rather that it functions solely as a repressor of *cydAB* expression.

Our data are consistent with previous findings that suggest that the cytochrome *o* oxidase complex encoded by *cyoABCDE* is the predominant respiratory enzyme under oxygen-rich growth conditions, while cytochrome *d* oxidase accumulates in cells as oxygen becomes limiting (22). Our data also indicate that even under oxygen-saturated growth conditions the level of the cytochrome *d* oxidase complex in the cell is not insignificant. Calculation of the number of molecules of each cytochrome oxidase in the cell (by the method of Grove and Gunsalus [7]) indicates that in aerobically grown cells the cytochrome *o* oxidase complex is present at a level of approximately 304 molecules per cell, whereas the cytochrome *d* oxidase complex is present at about 204 molecules per cell. Our calculations also indicate that under anaerobic growth conditions cytochrome *d* oxidase is the predominant species present (ca. 606 molecules per cell). Under these conditions the cytochrome *o* oxidase complex is present at only about 2 molecules per cell.

Assay of *cydA'*-*lacZ* expression in the *fnr* deletion strain indicates that there is potential for greater expression of this operon compared with that in the wild-type strain grown in the presence or absence of saturating oxygen. Possibly *cydAB* expression is maximal during microaerophilic growth, in which the cytochrome *d* oxidase complex, with its higher affinity for oxygen, would be better suited than the cytochrome *o* oxidase complex to support aerobic respiration. Under these conditions aerobic respiration with cytochrome *d* oxidase would be energetically more favorable than anaerobic respiration. The cell has apparently evolved a strategy of gene regulation to allow rapid adaption to the aerobic growth mode. A variety of obligate aerobes (e.g., *Rhizobium*, *Bradyrhizobium*, and *Azotobacter* species) and facultative microorganisms (i.e., the enteric bacteria) have dual cytochrome oxidase activities (4). Possibly the cellular levels of these dual cytochrome enzymes and their regulation by oxygen reflect the pattern seen in *E. coli* and might confer a superior ability to compete under various environmental conditions.

We found that *cyoABCDE* and *cydAB* expression varied somewhat with the carbon source used for cell growth. Expression was highest when cells were grown on nonfermentable carbon sources and was lowest when grown on glucose (Table 2). The repression effect by glucose on respiratory enzyme synthesis is similar to glucose repression of cytochrome *c* and cytochrome oxidase expression in *Saccharomyces cerevisiae* (8, 18). Possibly the respiratory enzymes are synthesized at a maximal rate when respiration is the only possible means of energy generation as opposed to conditions in which cells have the option of substrate-level phosphorylation.

The regulatory pattern of *cyoABCDE* and *cydAB* expression is consistent with a hierarchical global regulation of respiration-associated operons, whereby the compound that yields the greatest potential energy is used preferentially over other respiratory compounds whose reduction yields potentially less free energy (Fig. 3) (3, 11, 13, 26). The *narGHJI*, *dmsABC*, and *frdABCD* genes that encode the nitrate reductase, trimethylamine *N*-oxide:dimethyl sulfoxide reductase, and fumarate reductase enzymes, respectively, have been shown to be under positive control of the *fnr* gene product in response to anaerobiosis (3, 14, 26). We have shown in this study that the *fnr* regulator contributes to *cyoABCDE* and *cydAB* expression as a repressor anaerobically. Thus, Fnr appears to respond to anaerobiosis (presum-

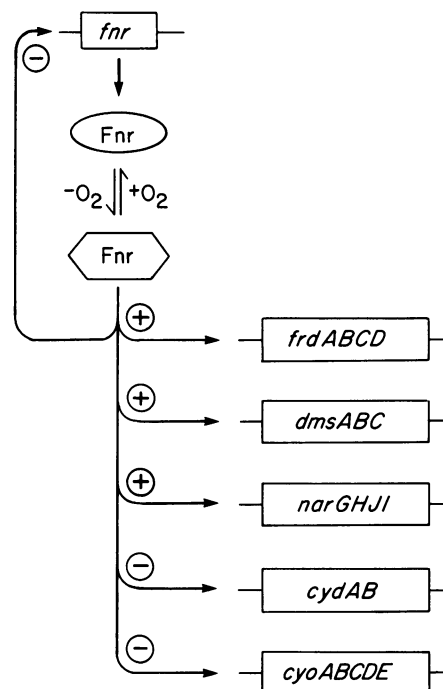


FIG. 3. Scheme for *fnr*-dependent global regulation of respiration-associated genes in *E. coli*. The genes encode the enzymes for the following enzymes: *frdABCD*, fumarate reductase; *narGHJI*, nitrate reductase; *dmsABC*, trimethylamine *N*-oxide:dimethyl sulfoxide reductase; *cydAB*, cytochrome *d* oxidase; and *cyoABCDE*, cytochrome *o* oxidase. Symbols indicate positive control (\oplus) (transcription activation) or negative control (\ominus) (transcriptional repression) of the indicated genes by the *fnr* gene product in response to anaerobiosis. Control by additional proposed regulators is not shown in this scheme.

ably through an oxygen- or redox-sensitive signal) to appropriately regulate the expression of the various terminal oxidoreductases in response to the availability of oxygen to insure maximum cellular energy generation (Fig. 3). It is interesting to note that Fnr may act as both an activator of anaerobic respiratory enzyme encoding genes and as a repressor of the genes of the aerobic pathway. Fnr has also been shown to be autoregulatory (14, 24, 28) and to repress expression of the NADH dehydrogenase II structural gene (25). Furthermore, Fnr functions over a considerable dynamic range. It mediates a 65-fold activation of gene expression in the case of *dmsABC* (3) as well as mediating a 30-fold repression for *cyoABCDE* expression (this study).

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